

US EPA ARCHIVE DOCUMENT

MRID No. 421534-01

DATA EVALUATION RECORD

1. **CHEMICAL:** Linuron.
Shaughnessey No. 035506.
2. **TEST MATERIAL:** DPX-Z326-198 (Linuron); N'-(3,4-dichlorophenyl)-N-methoxy-N-methyl-urea; CAS No. 330-55-2; Lot No. 90077284; 98.4% active ingredient.
3. **STUDY TYPE:** *Daphnia magna* Life-Cycle (21-day Renewal) Chronic Toxicity Test. Species Tested: *Daphnia magna*.
4. **CITATION:** Baer, K.N. 1991. Chronic Toxicity of DPX-Z326-198 (Linuron) to *Daphnia magna*. Laboratory Report No. 558-91. Prepared by Haskell Laboratory for Toxicology and Industrial Medicine, E.I. du Pont de Nemours and Co., Newark, DE. Submitted by E.I. du Pont de Nemours and Co., Newark, DE. EPA MRID No. 421534-01.

5. **REVIEWED BY:**

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7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a chronic, static-renewal toxicity test using the freshwater invertebrate, *Daphnia magna*. The MATC, based on the most sensitive parameter, daphnid survival, was >0.13 mg/l and <0.24 mg/l (geometric mean MATC = 0.18 mg/l) mean measured concentrations. The 21-day EC_{50} was 0.21 mg/l mean measured concentration.
8. **RECOMMENDATIONS:** N/A.
9. **BACKGROUND:**



10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

- A. Test Animals: *Daphnia magna* (<24 hours old) were obtained from in-house cultures. The cultures were housed in 1-l glass beakers containing 0.9 l of filtered dilution water and held at approximately 20°C. Neonates were collected from 19-to 26-day old adults for use in the test.

The adult daphnids were fed a combination of three green algae (*Ankistrodesmus falcatus*, *Chlamydomonas reinhardtii*, *Selenastrum capricornutum*) at a rate ranging from 50,000 to 200,000 cells/ml of each species three times weekly.

- B. Test System: The test vessels were 250-ml glass beakers containing 200 ml of test solution (6.8-cm depth). The test solutions were held between 19.2 and 20.1°C. The photoperiod was 16-hour light/8-hour dark with a light intensity of 129-172 lux.

The dilution water was well water which had flowed through aquaria containing fathead minnows and was filtered prior to use. The results of a characterization of the well water are given in Table 1 (attached).

A stock solution (100 mg/ml) was prepared in dimethylformamide (DMF). The stock was mixed with dilution water (final volume of 2 l) then distributed to the test beakers. The test solutions were not aerated during the test.

- C. Dosage: Twenty-one-day, static-renewal, life-cycle chronic toxicity test. Based on a rangefinding test and a previous definitive test, eight nominal concentrations (0.016, 0.041, 0.10, 0.26, 0.64, 1.6, 4.0, and 10 mg/l), a dilution water control, and a solvent control (0.1 ml DMF/l) were selected for the test.
- D. Design: Seven replicates per concentration contained one daphnid (for monitoring survival, growth, and reproduction) and 3 replicates per concentration contained five daphnids per beaker (for monitoring survival). Daphnids were randomly added to the control and test beakers. The solutions were renewed every Monday, Wednesday, and Friday. After each renewal, the

daphnids were fed the same algae used for culturing at a rate of 67,000 cells/ml of each species.

The survival and immobility of the daphnids were monitored daily. The presence of eggs in the brood pouch and the number of normal and immobile offspring produced were determined at each renewal on days 3, 5, 7, 10, 12, 14, 17, 19, and 21. The length of the daphnids used in the reproduction portion of the study was determined at test termination.

The dissolved oxygen concentration and pH were measured in one replicate of all test concentrations at test initiation, in the new and old solutions at each renewal, and at termination. Temperatures were measured daily with a mercury thermometer and continuously using a chart recorder. The conductivity, hardness, and alkalinity of a dilution water were measured at test initiation, weekly, and at the end of the test.

Samples of the fresh test solutions were taken on days 0, 2, 7, and 14, and samples of the old test solutions were taken on days 7, 14, and 21 for quantitative analysis of DPX-Z326-198 by HPLC.

- E. Statistics:** Survival was analyzed using a combination of Fisher's Exact test and the Cochran-Armitage trend test. The 21-day EC_{50} was determined using probit analysis. The total young per surviving adult female were analyzed using analysis of variance (ANOVA) and Dunnett's test. If no statistical difference was determined, control and solvent control data were pooled prior to analysis. Daphnid length, day of first brood, and number of young immobilized were analyzed using the Kruskal-Wallis test to determine overall differences between treatments and multiple comparison procedures (i.e., exact Mann-Whitney comparisons with Bonferroni adjustment) to determine differences of treatments from the control. The non-parametric procedures were employed because the results of Shapiro-Wilk's tests (on the residuals from the ANOVA) indicated the data were not normally distributed.

- 12. REPORTED RESULTS:** The mean measured concentrations were 0.016, 0.035, 0.13, 0.24, 0.51, 1.3, 3.4, and 8.4 mg/l (Table I, attached).

The total number of live and immobile young produced per adult in 21 days and the first day of reproduction were

significantly decreased at 3.4 mg/l and above (Table 4, attached). The length of surviving adults was significantly decreased at 1.3 mg/l and above. Survival data from replicates 1-7 (1 daphnid/replicate) and 8-10 (5 daphnids/replicate) were analyzed separately. The number of adults surviving to day 21 was significantly reduced at concentrations ≥ 3.4 and ≥ 0.24 mg/l, for replicates 1-7 and 8-10, respectively. The 21-day EC_{50} was 2.7 and 0.22 mg/l, for replicates 1-7 and 8-10, respectively. The author suggested that the difference in loading and food consumption between replicates 1-7 and 8-10 was probably responsible for the survival observed.

The pH of the test solutions ranged from 7.7 to 8.5. Dissolved oxygen concentration ranged from 8.1 to 9.7 mg/l. The temperature was reported as 19.2-20.1°C during the study. The total alkalinity, hardness, and conductivity of the dilution water was 77-80 mg/l as $CaCO_3$, 74-76 mg/l as $CaCO_3$, and 171-186 $\mu mhos/cm$, respectively, during the exposure.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

The 21-day no observed effect concentration (NOEC), based on adult survival in replicates 8-10, was 0.13 mg/l and the maximum acceptable toxicant concentration (MATC) was 0.18 mg/l.

Quality Assurance documentation was provided in the report. A GLP statement was included indicating adherence to USEPA GLP Regulations for FIFRA (40 CFR 160).

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Test Procedure: The test procedures were generally in accordance with the SEP and ASTM (1988), except for the following:

The dilution water was well water which had passed through a fish culture unit. The ammonia content may have been elevated and should have been checked prior to use.

ASTM states that each test concentration should be at least 50% of the next highest concentration. In this test the concentrations were approximately 40% of the next highest concentration.

Only the conductivity, hardness, and alkalinity of the dilution water were measured weekly. ASTM states that

these parameters must be measured in the control, low, medium, and high concentration test solutions weekly.

No transition periods between light and dark were used. Fifteen to thirty-minute transitions between light and dark are recommended.

The light intensity used during the test (129-172 lux) was lower than that recommended by the SEP (400-800 lux).

Test beakers should be covered to reduce evaporation. The report does not state if the beakers were covered.

Treatments must be randomly assigned to the test chambers. The report does not mention if the treatments were randomly assigned.

Length was measured to the nearest 0.1 mm; the SEP recommends measurement to the nearest 0.01 mm.

- B. **Statistical Analysis:** The reviewer used Fisher's Exact test to analyze the survival of the individually-tested daphnids (replicates 1-7 of each test level) and non-parametric multiple comparison procedures (Wilcoxon Rank Sum and Kruskal-Wallis) to analyze the survival of the daphnids tested in groups of 5 (replicates 8-10 of each test level). The results using the author's approach were more conservative than the reviewer's (see attached printouts 1 and 2). By visual inspection, survival was obviously affected at concentrations ≥ 0.24 mg/l. The EC_{50} was verified using the mortality results from replicates 8-10 and the moving average method (EPA's Toxanal program). The 21-day EC_{50} was 0.21 mg/l with a 95% C.I. of 0.13-0.32 mg/l (see attached printout 2a).

The reviewer calculated the number of young produced per adult reproductive day from information provided in Table 10 (attached). This data and the daphnid length data were analyzed using one-way ANOVA and William's or Bonferroni's tests (Toxstat Version 3.3). Reproduction and length of exposed daphnids were significantly lower than those of the solvent control at concentrations ≥ 0.51 and ≥ 1.3 mg/l, respectively (see attached printouts 3-6). There was no significant difference between the control and solvent control for length or reproduction.

- C. **Discussion/Results:** The disparity between the responses (mortality, length) of the individually-tested daphnids (replicates 1-7) and the daphnids tested in groups of 5 (replicates 8-10) greatly affects the MATC for this test. Historically, significant differences like those observed in this test have not been observed in other studies using the same test design.

The design used (7 replicates with one daphnid each and 3 replicates with 5 daphnids each) is recommended in the SEP. However, the differences in loading (one daphnid/200 ml of solution vs. 5 daphnids/200 ml of solution) must be addressed by the laboratory. The volume of food added to the containers possessing 5 adult daphnids should have been higher than that added to the beakers containing individual daphnids. The report states that the quantity of food offered was the same in all replicates. Control survival in all replicates was 100%. From notes in the appendix of the report, it is clear that the laboratory personnel had observed the daphnids in replicates 8-10 of all levels were smaller than those in replicates 1-7. In addition, a previous definitive test with this test material was unacceptable due to inadequate food supply.

This study is scientifically sound and meets the guideline requirements for a chronic, static-renewal toxicity test using the freshwater invertebrate, *Daphnia magna*. The MATC, based on the most sensitive parameter, daphnid survival, was >0.13 mg/l and <0.24 mg/l (geometric mean MATC = 0.18 mg/l) mean measured concentrations. The 21-day EC_{50} was 0.21 mg/l mean measured concentration.

- D. **Adequacy of the Study:**

- (1) **Classification:** Core.
- (2) **Rationale:** N/A.
- (3) **Repairability:** N/A.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 03-18-92.

REFERENCES: ASTM. 1988. Standard Guide for Conducting Renewal Life-Cycle Toxicity Tests with *Daphnia magna*. E 1193-87.

TABLE 1

Chemical Characteristics of Haskell Laboratory Well Water¹
(MR 9118-001)

Parameter	Concentration	Parameter	Concentration
BOD ² , mg/L	<2	Lead ⁵ , mg/L	<0.005
COD, mg/L	13	Magnesium, mg/L	3.5
DOC ³ , mg/L	1.5	MBAS/LAS, mg/L	<0.05
TOC ⁴ , mg/L	<0.5	Mercury, mg/L	<0.00020
Kjeldahl N, mg/L	0.2	Nickel, mg/L	<0.020
Ammonia N, mg/L	<0.05	Nitrite ⁶ , mg/L	<0.1
Turbidity, NTU	<1.0	Nitrate ⁶ , mg/L	1.6
Phenolics, mg/L	<0.050	Phosphate ⁶ , mg/L	<0.1
Color, Co/Pt	<5	Potassium, mg/L	1.8
Solids		Selenium, mg/L	<0.005
total suspended, mg/L	<2	Silver, mg/L	<0.010
Aluminum, mg/L	<0.100	Sodium, mg/L	8.1
Antimony, mg/L	<0.060	Sulfate ⁶ , mg/L	4.8
Arsenic, mg/L	<0.010	Sulfide, mg/L	<0.05
Beryllium, mg/L	<0.001	Thallium, mg/L	<0.01
Boron ⁵ , mg/L	<0.020	Zinc ⁵ , mg/L	<0.020
Cadmium, mg/L	<0.002	Volatile priority	
Calcium, mg/L	25.0	pollutants	ND ⁸
Chloride ⁶ , mg/L	6.3	Acid extractable	
Chromium, mg/L	<0.010	priority pollutants	ND ⁸
Cobalt, mg/L	<0.020	Base/neutral	
Copper, mg/L	<0.010	priority pollutants	ND ⁸
Cyanide, mg/L	<0.025	Pesticides/PCBs	ND ⁸
Iron ⁶ , mg/L	0.053	Organophosphate	
Fluoride ⁶ , mg/L	<0.1	pesticides ⁷ , µg/L	<0.50

¹Date of analyses 17 October 1990 unless indicated otherwise, analyses performed at Environmental Testing and Certification Corporation, Edison, New Jersey; ²Date of analyses 13 December 1990, analyses analysis performed at Environmental Testing and Certification Corporation, Edison, New Jersey; ³Mean value; ⁴Date of analyses 24 July 1990, performed at Du Pont Engineering Test Center, Newark, Delaware; ⁵Below the Published Method Detection Limit (BMDL); ⁶Date of analyses 9 November 1990, analyses performed at Du Pont Chemicals, Jackson Laboratory, Deepwater, New Jersey; ⁷Date of analyses 23 October 1990, analyses performed at Hazleton Laboratories, Inc., Madison, WI; ⁸None detected.

TABLE 4

Summary of Data for Daphnia magna Exposed to DPX-Z326-198
for 21 Days in a Static, Renewal Test
(MR 9118-001)

Mean, Measured Concentrations (mg/L)	Mean (Standard Deviation)					
	Percent Adult Survival		1st Reprod Day ³	Live Young ⁴	Immobile Young ⁵	Length (mm) ⁶
	Rep 1-7 ¹	Rep 8-10 ²				
Water Control	100	100	9.7	171 (42)	0 (0.0)	4.6 (0.28)
DMF Control	100	100	10	166 (41)	0 (0.0)	4.6 (0.15)
0.016	100	100	9.9	145 (21)	0 (0.0)	4.5 (0.17)
0.035	100	87	10	152 (22)	0 (0.0)	4.5 (0.17)
0.13	100	93	10	135 (27)	0 (0.0)	4.5 (0.14)
0.24	100	13*	9.9	151 (40)	0 (0.0)	4.4 (0.21)
0.51	71	47*	10	135 (30)	0 (0.0)	4.5 (0.18)
1.3	100	0*	9.3	141 (40)	0 (0.0)	4.3* (0.22)
3.4	57*	0*	14*	53* (34)	13* (3.5)	3.0* (0.39)
8.4	0*	0*	D ⁷	D	D	D

¹One fish per replicate; ²Five fish per replicate; ³First day of reproduction; ⁴Sum of live young produced per surviving female in 21 days; ⁵Sum of immobilized young produced per surviving adult in 21 days (data obtained from replicates 1-7); ⁶Data obtained from replicates 1-7; ⁷D means all daphnids were dead, i.e., no data available. *Significantly different from controls at the 0.05 level.

TABLE 10

Reproduction and Length Data at Test Conclusion for Daphnia magna
Exposed to DPX-Z326-198 for 21 Days in a Static, Renewal Test
(MR 9118-001)

Mean, Measured Concentrations (mg/L)	Rep ¹	1st Reprod Day ²	<i>Number of Reproductive days</i>	Total Live Young ³	<i>Live young per adult Reproductive day</i>	Immobile Young ⁴	Length (mm)
Water Control							
	1	10	9 11	212	19.3	0	4.6
	2	8	12 13	193	14.8	0	4.7
	3	9	12	195	16.3	0	4.9
	4	10	9 11	163	14.8	0	4.2
	5	10	9 11	88	8.0	0	4.2
	6	11	10	194	19.4	0	4.8
	7	10	11	155	14.1	0	4.7
Solvent Control							
	1	11	10	198	19.8	0	4.7
	2	11	10	152	15.2	0	4.4
	3	9	12	221	18.4	0	4.7
	4	11	10	106	10.6	0	4.4
	5	10	11	188	17.1	0	4.8
	6	10	11	125	11.4	0	4.6
	7	10	11	172	15.6	0	4.6
0.016							
	1	10	11	111	10.1	0	4.6
	2	10	11	136	12.4	0	4.6
	3	10	11	178	16.2	0	4.6
	4	9	12	151	12.6	0	4.6
	5	10	11	150	13.6	0	4.2
	6	10	11	130	11.8	0	4.7
	7	10	11	157	14.3	0	4.4

¹Replicate (1 daphnid per replicate); ²First day of reproduction; ³Sum of live young produced per surviving adult in 21 days (data obtained for replicates 1-7); ⁴Sum of Immobile young produced per surviving adult in 21 days (data obtained for replicates 1-7).

TABLE 10, Continued

Reproduction and Length Data at Test Conclusion for Daphnia magna
Exposed to DPX-Z326-198 for 21 Days in a Static, Renewal Test
(MR 9118-001)

Mean, Measured Concentrations (mg/L)	Rep ¹	1st Reprod Day ²		Total Live Young ³		Immobile Young ⁴	Length (mm)
0.035	1	10	"	181	16.5	0	4.5
	2	10	"	158	14.4	0	4.4
	3	10	"	148	13.5	0	4.6
	4	10	"	119	10.8	0	4.7
	5	10	"	148	13.5	0	4.2
	6	10	"	132	12	0	4.6
	7	10	"	176	16	0	4.6
0.13	1	10	"	118	10.7	0	4.2
	2	10	"	107	9.7	0	4.5
	3	10	"	148	13.5	0	4.5
	4	11	"	172	15.6	0	4.6
	5	10	"	123	11.2	0	4.4
	6	10	"	108	9.8	0	4.6
	7	10	"	166	15.1	0	4.5
0.24	1	10	"	136	12.4	0	4.4
	2	10	"	119	10.8	0	4.6
	3	10	"	202	18.4	0	4.5
	4	10	"	132	12	0	4.4
	5	9	12	194	17.6 16.2	0	4.0
	6	10	"	97	8.8	0	4.6
	7	10	"	175	15.9	0	4.6

¹Replicate (1 daphnid per replicate); ²First day of reproduction; ³Sum of live young produced per surviving adult in 21 days (data obtained for replicates 1-7); ⁴Sum of Immobile young produced per surviving adult in 21 days (data obtained for replicates 1-7);.

TABLE 10, Continued

Reproduction and Length Data at Test Conclusion for Daphnia magna
Exposed to DPX-Z326-198 for 21 Days in a Static, Renewal Test
(MR 9118-001)

Mean, Measured Concentrations (mg/L)	Rep ¹	1st Reprod Day ²	Total Live Young ³	Immobile Young ⁴	Length (mm)
0.51					
	1	10 4	37 ⁵ 9.3	0	D ⁶
	2	9 12	174 14.5	0	4.6
	3	9 5	21 ⁵ 4.2	0	D
	4	10 "	139 12.6	0	4.4
	5	10 "	148 13.5	0	4.6
	6	12 9	122 13.6	0	4.2
	7	10 "	93 8.5	0	4.6
1.3					
	1	10 "	120 10.9	0	4.2
	2	8 13	186 14.3	0	4.3
	3	10 "	128 11.6	0	3.9
	4	10 "	118 10.7	0	4.6
	5	10 "	85 7.7	0	4.2
	6	8 13	196 15.1	0	4.4
	7	9 12	157 13.1	0	4.4
3.4					
	1	D 0	D 0	D	D
	2	12 9	80 8.9	0	2.6
	3	12 9	81 9.0	9	3.4
	4	12 9	38 4.2	4	3.3
	5	D 0	D 0	D	D
	6	18 3	11 3.7	0	2.8
	7	D 0	D 0	D	D

¹Replicate (1 daphnid per replicate); ²First day of reproduction; ³Sum of live young produced per surviving adult in 21 days (data obtained for replicates 1-7); ⁴Sum of Immobile young produced per surviving adult in 21 days (data obtained for replicates 1-7); ⁵Data not included in analysis because daphnids did not survive to day 21; ⁶D means daphnid had previously died and no data available.

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TABLE 10, Continued

Reproduction and Length Data at Test Conclusion for Daphnia magna
Exposed to DPX-Z326-198 for 21 Days in a Static, Renewal Test
(MR 9118-001)

Mean, Measured Concentrations (mg/L)	Rep ¹	1st Reprod Day ²	Total Live Young ³	Immobile Young ⁴	Length (mm)
8.4	1	D ⁵	D	D	D
	2	D	D	D	D
	3	D	D	D	D
	4	D	D	D	D
	5	D	D	D	D
	6	D	D	D	D
	7	D	D	D	D

¹Replicate (1 daphnid per replicate); ²First day of reproduction; ³Sum of young produced per surviving adult in 21 days (data obtained for replicates 1-7); ⁴Sum of Immobile young produced per surviving adult in 21 days (data obtained for replicates 1-7); ⁵D means daphnid had previously died and no data table.

Table I

Measured Concentrations of Active Ingredient in DPX-Z326-198 Test Solutions

Nominal Concentration ^a (mg/L)	Measured Test Concentration (mg/L)							
	Day 0	Day 2 new	Day 7 ^c old	Day 7 ^c new	Day 14 old	Day 14 new	Day 21	Average ^b
0.0 Control 1A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0
0.0 Control 2A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0
0.016 Control 1A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0
0.016 Control 2A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0
0.016 1A	0.014	0.015	0.014	0.013	0.021	0.016	0.020	0.016
0.016 2A	0.012	0.015	0.014	0.017	0.018	0.018	0.016	0.016
0.041 1A	0.040	0.036	0.034	0.026	0.037	0.031 ^d	0.040	0.035
0.041 2A	0.039	0.036	0.036	0.030	0.037	0.030 ^d	0.040	0.035
0.10 1A	0.146	0.097	0.143	0.133	0.099	0.152 ^d	0.154	0.13
0.10 2A	0.142	0.092	0.139	0.128	0.099	0.144 ^d	0.154	0.13
0.26 1A	0.209	0.018 ^e	0.255	0.206	0.245	0.246	0.259	0.24
0.26 2A	0.237	0.015 ^e	0.231	0.203	0.248	0.234	0.266	0.24
0.64 1A	0.530	0.548	0.428	0.508	0.557	0.547	0.556	0.52
0.64 2A	0.447	0.474 ^d	0.436	0.458	0.557	0.548	0.567	0.50
1.6 1A	1.30	1.28	1.19	1.20	1.33	1.23	1.26	1.3
1.6 2A	1.21	1.28	1.19	1.20	1.34	1.24	1.27	1.2
4.0 1A	3.57	3.37	3.19	3.62	3.41	3.04 ^d	3.50	3.4
4.0 2A	3.45	3.32	3.31	3.16	3.58	2.86 ^d	3.58	3.3
10.0 1A	8.68	8.43	---	---	---	---	---	8.6
10.0 2A	8.32	7.78	---	---	---	---	---	8.1

Contains 98.4% a.i. ^b Average reported to two significant figures. ^c The results of the re-analysis of 0.016 and 0.041 mg/L and of the back-up 0.10 and 0.26 mg/L test solutions are reported due to error in the original calibration. ^d Analysis of back-up samples confirmed original results. Original results reported. ^e Analysis of back-up confirmed original results, probable dilution of 0.016 mg/L test solution. Not used to calculate average.

Circled values are less than 50% of the 272 highest measured concentration

421534-01, LINURON, ANALYSIS OF MORTALITY IN REPLICATES CONTAINING INDIVIDUAL DAPHNIDS

SUMMARY OF FISHERS EXACT TESTS

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG (P=.05)
	CONTROL	7	0	
1	0.016 mg/l	7	0	
2	0.035	7	0	
3	0.13	7	0	
4	0.24	7	0	
5	0.51	7	2	
6	1.3	7	0	
7	3.4	7	3	
8	8.4	7	7	*

TITLE: 421534-01, linuron, daphnid survival in replicates containing groups of five daphnids.

FILE: a:42153401.dtl

TRANSFORM: ARC SINE(SQUARE ROOT(Y))

NUMBER OF GROUPS: 9

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	solvent control	1	1.0000	1.3453
1	solvent control	2	1.0000	1.3453
1	solvent control	3	1.0000	1.3453
2	0.016 mg/l	1	1.0000	1.3453
2	0.016 mg/l	2	1.0000	1.3453
2	0.016 mg/l	3	1.0000	1.3453
3	0.035	1	1.0000	1.3453
3	0.035	2	0.8000	1.1071
3	0.035	3	0.8000	1.1071
4	0.13	1	1.0000	1.3453
4	0.13	2	1.0000	1.3453
4	0.13	3	0.8000	1.1071
5	0.24	1	0.0000	0.2255
5	0.24	2	0.2000	0.4636
5	0.24	3	0.2000	0.4636
6	0.51	1	0.4000	0.6847
6	0.51	2	1.0000	1.3453
6	0.51	3	0.0000	0.2255
7	1.3	1	0.0000	0.2255
7	1.3	2	0.0000	0.2255
7	1.3	3	0.0000	0.2255
8	3.4	1	0.0000	0.2255
8	3.4	2	0.0000	0.2255
8	3.4	3	0.0000	0.2255
9	8.4	1	0.0000	0.2255
9	8.4	2	0.0000	0.2255
9	8.4	3	0.0000	0.2255

421534-01, linuron, daphnid survival

File: a:42153401.dtl

Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro Wilks test for normality

Data FAIL normality test. Try another transformation.

Hartley test for homogeneity of variance

Bartlett's test for homogeneity of variance

These two tests can not be performed because at least one group has zero variance.

Data FAIL to meet homogeneity of variance assumption.

Additional transformations are useless.

421534-01, linuron, daphnid survival (Replicates 8-10, 5 daphnids per Replicate)

File: a:42153401.dtl

Transform: ARC SINE(SQUARE ROOT(Y))

WILCOXON RANK SUM TEST W/ BONFERRONI ADJUSTMENT - Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	REPS	SIG
1	solvent control	1.345				
2	0.016 mg/l	1.345	10.50	None	3	
3	0.035	1.187	7.50	None	3	
4	0.13	1.266	9.00	None	3	
5	0.24	0.384	6.00	None	3	
6	0.51	0.752	7.50	None	3	

Critical values use k = 5, are 1 tailed, and alpha = 0.05

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2 (p=0.05)

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP					
				0	0	0	0	0	0
				5	6	3	4	1	2
5	0.24	0.384	0.133	\					
6	0.51	0.752	0.467	.	\				
3	0.035	1.187	0.867	.	.	\			
4	0.13	1.266	0.933	.	.	.	\		
1	solvent control	1.345	1.000	\	
2	0.016 mg/l	1.345	1.000	\

* = significant difference (p=0.05)

Table q value (0.05,6) = 2.936

. = no significant difference

SE = 3.956

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RIFICI LINURON DAPHNIA MAGNA 03-18-92

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
8.399999	15	15	100	3.051758E-03
3.051758E-03				
3.4	15	15	100	3.051758E-03
1.3	15	15	100	3.051758E-03
.51	15	8	53.33334	50
.24	15	13	86.66666	.3692627
.13	15	1	6.666667	4.882813E-02
.035	15	2	13.33333	.3692627
.016	15	0	0	3.051758E-03

LMR
3/18/92

THE BINOMIAL TEST SHOWS THAT .13 AND 1.3 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS .1828931

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
7	5.912288E-02	0.2083465	.1284058 - 0.3170118

LMR 3/18/92

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	.5128495	3.175591	4.073799E-03

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 2.005625
95 PERCENT CONFIDENCE LIMITS = .5693264 AND 3.441923

LC50 = .2192542
95 PERCENT CONFIDENCE LIMITS = .0601257 AND .6687374

LC10 = .0510186
95 PERCENT CONFIDENCE LIMITS = 7.73254E-04 AND .1307096

TITLE: 421531-01, linuron, # young/adult reproductive day

FILE: a:42153401.dt2

TRANSFORM: NO TRANSFORMATION

NUMBER OF GROUPS: 9

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	solvent control	1	19.8000	19.8000
1	solvent control	2	15.2000	15.2000
1	solvent control	3	18.4000	18.4000
1	solvent control	4	10.6000	10.6000
1	solvent control	5	17.1000	17.1000
1	solvent control	6	11.4000	11.4000
1	solvent control	7	15.6000	15.6000
2	dilut. control	1	19.3000	19.3000
2	dilut. control	2	14.8000	14.8000
2	dilut. control	3	16.3000	16.3000
2	dilut. control	4	14.8000	14.8000
2	dilut. control	5	8.0000	8.0000
2	dilut. control	6	19.4000	19.4000
2	dilut. control	7	14.1000	14.1000
3	0.016 mg/l	1	10.1000	10.1000
3	0.016 mg/l	2	12.4000	12.4000
3	0.016 mg/l	3	16.2000	16.2000
3	0.016 mg/l	4	12.6000	12.6000
3	0.016 mg/l	5	13.6000	13.6000
3	0.016 mg/l	6	11.8000	11.8000
3	0.016 mg/l	7	14.3000	14.3000
4	0.035	1	16.5000	16.5000
4	0.035	2	14.4000	14.4000
4	0.035	3	13.5000	13.5000
4	0.035	4	10.8000	10.8000
4	0.035	5	13.5000	13.5000
4	0.035	6	12.0000	12.0000
4	0.035	7	16.0000	16.0000
5	0.13	1	10.7000	10.7000
5	0.13	2	9.7000	9.7000
5	0.13	3	13.5000	13.5000
5	0.13	4	15.6000	15.6000
5	0.13	5	11.2000	11.2000
5	0.13	6	9.8000	9.8000
5	0.13	7	15.1000	15.1000
6	0.24	1	12.4000	12.4000
6	0.24	2	10.8000	10.8000
6	0.24	3	18.4000	18.4000
6	0.24	4	12.0000	12.0000
6	0.24	5	16.2000	16.2000
6	0.24	6	8.8000	8.8000
6	0.24	7	15.9000	15.9000
7	0.51	1	9.3000	9.3000
7	0.51	2	14.5000	14.5000
7	0.51	3	4.2000	4.2000
7	0.51	4	12.6000	12.6000
7	0.51	5	13.5000	13.5000
7	0.51	6	13.6000	13.6000
7	0.51	7	8.5000	8.5000
8	1.3	1	10.9000	10.9000
8	1.3	2	14.3000	14.3000
8	1.3	3	11.6000	11.6000
8	1.3	4	10.7000	10.7000
8	1.3	5	7.7000	7.7000
8	1.3	6	15.1000	15.1000
8	1.3	7	13.1000	13.1000
9	3.4	1	8.9000	8.9000
9	3.4	2	9.0000	9.0000
9	3.4	3	4.2000	4.2000
9	3.4	4	3.7000	3.7000

421531-01, linuron, # young/adult reproductive day
 File: a:42153401.dt2 Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies
 Data PASS normality test. Continue analysis.

Bartlett's test for homogeneity of variance
 Data PASS homogeneity test at 0.01 level. Continue analysis.

t-test of Solvent and Blank Controls

Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CTRL) MEAN =	15.4429	CALCULATED t VALUE =	0.1027
GRP2 (BLANK CTRL) MEAN =	15.2429	DEGREES OF FREEDOM =	12
DIFFERENCE IN MEANS =	0.2000		

TABLE t VALUE (0.05 (2),12) = 2.179 NO significant difference at alpha=0.05
 TABLE t VALUE (0.01 (2),12) = 3.055 NO significant difference at alpha=0.01

WILLIAMS TEST (Isotonic regression model)

TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	solvent control	7	15.443	15.443	15.443
2	dilut. control	7	15.243	15.243	15.243
3	0.016 mg/l	7	13.000	13.000	13.407
4	0.035	7	13.814	13.814	13.407
5	0.13	7	12.229	12.229	12.864
6	0.24	7	13.500	13.500	12.864
7	0.51	7	10.886	10.886	11.400
8	1.3	7	11.914	11.914	11.400
9	3.4	4	6.450	6.450	6.450

WILLIAMS TEST (Isotonic regression model)

TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
solvent control	15.443				
dilut. control	15.243	0.125		1.68	k= 1, v=51
0.016 mg/l	13.407	1.269		1.76	k= 2, v=51
0.035	13.407	1.269		1.79	k= 3, v=51
0.13	12.864	1.607		1.80	k= 4, v=51
0.24	12.864	1.607		1.80	k= 5, v=51
0.51	11.400	2.520	*	1.81	k= 6, v=51
1.3	11.400	2.520	*	1.81	k= 7, v=51
3.4	6.450	4.779	*	1.81	k= 8, v=51

s = 3.002

Note: df used for table values are approximate when v > 20.

TITLE: 421534-01, linuron, adult daphnid length

FILE: a:42153401.dt3

TRANSFORM: NO TRANSFORMATION

NUMBER OF GROUPS: 9

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	solvent control	1	4.7000	4.7000
1	solvent control	2	4.4000	4.4000
1	solvent control	3	4.7000	4.7000
1	solvent control	4	4.4000	4.4000
1	solvent control	5	4.8000	4.8000
1	solvent control	6	4.6000	4.6000
1	solvent control	7	4.6000	4.6000
2	dilut. control	1	4.6000	4.6000
2	dilut. control	2	4.7000	4.7000
2	dilut. control	3	4.9000	4.9000
2	dilut. control	4	4.2000	4.2000
2	dilut. control	5	4.2000	4.2000
2	dilut. control	6	4.8000	4.8000
2	dilut. control	7	4.7000	4.7000
3	0.016 mg/l	1	4.6000	4.6000
3	0.016 mg/l	2	4.6000	4.6000
3	0.016 mg/l	3	4.6000	4.6000
3	0.016 mg/l	4	4.6000	4.6000
3	0.016 mg/l	5	4.2000	4.2000
3	0.016 mg/l	6	4.7000	4.7000
3	0.016 mg/l	7	4.4000	4.4000
4	0.035	1	4.5000	4.5000
4	0.035	2	4.4000	4.4000
4	0.035	3	4.6000	4.6000
4	0.035	4	4.7000	4.7000
4	0.035	5	4.2000	4.2000
4	0.035	6	4.6000	4.6000
4	0.035	7	4.6000	4.6000
5	0.13	1	4.2000	4.2000
5	0.13	2	4.5000	4.5000
5	0.13	3	4.5000	4.5000
5	0.13	4	4.6000	4.6000
5	0.13	5	4.4000	4.4000
5	0.13	6	4.6000	4.6000
5	0.13	7	4.5000	4.5000
6	0.24	1	4.4000	4.4000
6	0.24	2	4.6000	4.6000
6	0.24	3	4.5000	4.5000
6	0.24	4	4.4000	4.4000
6	0.24	5	4.0000	4.0000
6	0.24	6	4.6000	4.6000
6	0.24	7	4.6000	4.6000
7	0.51	1	4.6000	4.6000
7	0.51	2	4.4000	4.4000
7	0.51	3	4.6000	4.6000
7	0.51	4	4.2000	4.2000
7	0.51	5	4.6000	4.6000
8	1.3	1	4.2000	4.2000
8	1.3	2	4.3000	4.3000
8	1.3	3	3.9000	3.9000
8	1.3	4	4.6000	4.6000
8	1.3	5	4.2000	4.2000
8	1.3	6	4.4000	4.4000
8	1.3	7	4.4000	4.4000
9	3.4	1	2.6000	2.6000
9	3.4	2	3.4000	3.4000
9	3.4	3	3.3000	3.3000
9	3.4	4	2.8000	2.8000

421534-01, linuron, adult daphnid length
 File: a:42153401.dt3 Transform: NO TRANSFORM

t-test of Solvent and Blank Controls

Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CTRL) MEAN =	4.6000	CALCULATED t VALUE =	0.1187
GRP2 (BLANK CTRL) MEAN =	4.5857	DEGREES OF FREEDOM =	12
DIFFERENCE IN MEANS =	0.0143		

TABLE t VALUE (0.05 (2),12) = 2.179 NO significant difference at alpha=0.05
 TABLE t VALUE (0.01 (2),12) = 3.055 NO significant difference at alpha=0.01

Chi-square test for normality: actual and expected frequencies
 Data PASS normality test. Continue analysis.

Bartlett's test for homogeneity of variance
 Data PASS homogeneity test at 0.01 level. Continue analysis.

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	8	8.455	1.057	23.465
Within (Error)	49	2.207	0.045	
Total	57	10.662		

Critical F value = 2.18 (0.05,8,40)
 Since F > Critical F REJECT Ho:All groups equal

BONFERRONI T-TEST - TABLE 1 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	solvent control	4.600	4.600		
2	dilut. control	4.586	4.586	0.126	
3	0.016 mg/l	4.529	4.529	0.630	
4	0.035	4.514	4.514	0.756	
5	0.13	4.471	4.471	1.133	
6	0.24	4.443	4.443	1.385	
7	0.51	4.480	4.480	0.966	
8	1.3	4.286	4.286	2.771	*
9	3.4	3.025	3.025	11.840	*

Bonferroni T table value = 2.62 (1 Tailed Value, P=0.05, df=40,8)

BONFERRONI T-TEST - TABLE 2 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	solvent control	7			
2	dilut. control	7	0.297	6.5	0.014
3	0.016 mg/l	7	0.297	6.5	0.071
4	0.035	7	0.297	6.5	0.086
5	0.13	7	0.297	6.5	0.129
6	0.24	7	0.297	6.5	0.157
7	0.51	5	0.325	7.1	0.120
8	1.3	7	0.297	6.5	0.314
9	3.4	4	0.348	7.6	1.575